The Lack of Accumulation of Senile Plaques or Amyloid Burden in Alzheimer's Disease Suggests a Dynamic Balance Between Amyloid Deposition and Resolution

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Abstract. Aβ, a nearly insoluble peptide, is generally assumed to irreversibly deposit and accumulate as senile plaques (SP) during the course of Alzheimer's disease (AD). We have studied parietal neocortex of normal elderly subjects, AD patients, and elderly Down syndrome (DS) patients to determine whether Aβ accumulates with age or with increasing duration of illness. We measured the number, size distribution, and total area (amyloid burden) of Aβ immunoreactive deposits using computerized image analysis techniques. We found far fewer SP in normal control subjects than in AD patients, who in turn have fewer SP than elderly DS patients. No measure of Aβ correlated with age in the control subjects, nor duration of illness in AD or DS patients. These data indicate that Aβ may not continue to accumulate during these disease processes and support the view that the amount of Aβ observed at autopsy may reflect competing processes of deposition and resolution of amyloid.

Key Words: 10153; Aβ, βA4; Alzheimer's disease; Amyloid burden; Senile plaques.

INTRODUCTION

Senile plaques (SP) appear in the brain in Alzheimer’s disease (AD) and, to a lesser extent, in normal aging. The major biochemical component of SP is Aβ (βA4) protein, a small fragment derived from larger beta amyloid precursors (APP). The evidence that Aβ and APP are important in the pathogenesis of AD is strong. Mutations in the APP gene have been linked to the development of familial AD (1), and it has been suggested that the predisposition of Down syndrome (DS) individuals to early AD is due to overproduction of APP due to triplication of the APP gene (2). Moreover, Aβ has been shown to be a potentially neurotoxic substance (3–6). Paradoxically, however, in our recent study (7) and in reports of others (8–12), the number of SP correlates very poorly or not at all with duration of illness or severity of dementia in AD.

There are several possible explanations for the discrepancy between histologic and clinical data. The lack of correlation could be due, at least in part, to technical problems of counting SP. Senile plaques can be visualized using a variety of silver stains, histological stains that recognize amyloid, and by immunohistochemistry specific for the Aβ peptide. Each procedure has a different level of sensitivity, and the sensitivity may even vary substantially between laboratories (13, 14). Moreover, these staining procedures also provide slightly different images of the shape and size of SP, and descriptive taxonomies of SP that rely on morphological classification are somewhat dependent on the stain used.

An alternative possibility is that there is an error in one of the underlying assumptions of the hypothesis that SP accumulate with increased severity of disease. It is widely assumed that, since Aβ is nearly insoluble in physiological solution, once Aβ is deposited in the neuropil it remains and accumulates. However, if SP undergo a dynamic kinetic process of deposition and resolution, the number observed at autopsy would reflect a steady state, rather than a summation, of Aβ deposition. We tested the hypothesis that Aβ accumulates in AD.

We reasoned that a measure of the amount of total amyloid deposited, rather than the number of SP, might correlate better with clinical parameters. A measure of amyloid deposition may also be preferable because SP can vary in size considerably, and immunohistochemical techniques for Aβ reveal additional morphologic varieties of amyloid deposits including diffuse plaques and dot-like structures, leading to difficulty and perhaps arbitrary decisions as to how (and whether) to count these structures in SP counts. We used a computerized image analysis system to count SP, measure their size, and determine the sum of the area of all Aβ immunoreactive structures ("amyloid burden") in a series of normal elderly individuals and patients with AD or DS. We found that there is no increase in SP number, size, or amyloid burden with increasing duration of dementia. If we assume that Aβ continues to be generated, we suggest that this result implies a mechanism of resolution or resorption of Aβ during the course of the disease.

MATERIALS AND METHODS

Cases

The case material consisted of the inferior temporal gyrus of 15 AD patients, 25 age-compatible, nondemented controls (of whom 11 had Aβ deposits), and five elderly DS patients with AD (Table 1). All AD cases were individuals who had been
followed in the Memory Disorder Clinic at Massachusetts General Hospital. During life, all had a clinical diagnosis of AD and did not have any complicating medical conditions. The clinical diagnosis was confirmed at autopsy, and there were no other causes for dementia identified. Duration of illness in each individual was established by a neurologist based upon information provided by family members at the initial visit. Duration of illness, rather than a neuropsychometric test such as the Blessed dementia score, was chosen as the clinical parameter because neuropsychological tests have a floor effect when patients are no longer able to perform the task, thus eliminating from study individuals with advanced disease. The normal elderly subjects were selected from the autopsy population at Massachusetts General Hospital. The selection criteria are detailed elsewhere (15), but briefly these individuals are presumed to have been nondemented before they died following an acute illness because: 1) they had lived independently before entering the hospital; 2) they were examined by a psychiatrist, neurologist, or internist during the hospitalization preceding death and no cognitive impairment had been noted; 3) they died of non-neuropathologic conditions; and 4) they had an unremarkable general neuropathological examination, and specifically did not meet Khachaturian criteria for AD (16) or CERAD criteria (14) for possible AD. These 25 individuals were selected for study from 312 consecutive autopsies screened by these criteria. Down syndrome individuals had classic clinical stigmata of the disease, and although we do not have detailed clinical information, they all had marked AD changes seen at neuropathological examination.

### Table 1: Patient Characteristics

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Age</th>
<th>#SP/mm³</th>
<th>Amyloid burden*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>11</td>
<td>75.7 ± 3.4 (64–103)**</td>
<td>17 ± 5 (1–56)</td>
<td>1.4 ± 0.4 (0.1–3.8)</td>
</tr>
<tr>
<td>(w/o SP)</td>
<td>2</td>
<td>68.0 ± 3.0 (48–65)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Alzheimer’s disease</td>
<td>15</td>
<td>79.3 ± 1.7 (66–90)</td>
<td>156 ± 15 (53–256)†</td>
<td>6.0 ± 0.5 (3.8–9.1)†</td>
</tr>
<tr>
<td>Down syndrome</td>
<td>5</td>
<td>59.8 ± 1.6 (57–65)</td>
<td>252 ± 31 (192–360)$</td>
<td>15.6 ± 1.7 (10.5–19.6)$</td>
</tr>
</tbody>
</table>

* Amyloid burden refers to the percent of total cortical area covered by β/44 immunoreactivity.
** Data are presented as mean ± SE (range).
† Significant at p < 0.001 compared to controls and to Down syndrome.
§ Significant at p < 0.001 compared to controls and to Alzheimer’s disease.

We examined the inferior temporal gyrus (Brodmann area 20) because our earlier studies showed that it is one of the regions

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**Fig. 1.** Aβ immunostaining of temporal neocortex in AD using monoclonal antibody 10D5. The dotted box shows the size of a field 870 μm wide by the depth of the cortex. Magnification bar = 200 μm.
most severely and consistently affected by SP in AD and in aging (7, 15, 19). Senile plaque quantitation was carried out using a Bioquant Microquant image analysis system (Knoxville, TN), which is an operator-interactive image analysis device that is linked to stage x-y encoders and utilizes a Dage MTI CCD72 video camera system with a Leitz Aristoplan microscope. Images are captured, SP identified by grey scale thresholding, artifacts and vessel-associated amyloid staining deleted, and adjacent or overlapping structures separated by manual editing. Amyloid plaque subtypes were not specifically identified. A strip of cortex approximately 1 cm lateral to the collateral sulcus which was 870 μm wide by the depth of the cortex was selected. The area of the strip, the number of SP, the area of each SP, and the sum of the areas of SP divided by total area (amyloid burden) were calculated.

Statistical analysis was by ANOVA using the Statview II pro-

gram (Abacus Concepts), and post hoc comparisons used the Fischer protected least significant difference test. Significance was assigned at 0.05%.

RESULTS

Normal Aging

Our screening criteria eliminated from this subject group any individuals who met conventional criteria for AD based on a Bielschowsky silver stain (16). Nonetheless, when using amyloid immunohistochemistry on thick sections we found that 14 of the 25 normal elderly individuals had Aβ deposits in at least one of 20 cytoarchitectural fields examined (15), and 11 had Aβ deposits in neocortical area 20. Of these 11, five had fewer than five SP/mm², and six had above ten SP/mm². These results suggest that occasional presumably normal individuals can have a moderate amount of amyloid deposition. Senile plaque counts ranged from 1–56 SP/mm², and the amyloid burden among the 11 cases ranged from 0.1–3.8%. Senile plaque size averaged 950 ± 268 μm² (x ± SE). Thus, in those normal aging individuals where there were SP, the total number tended to be small but each individual SP tended to be large as compared to SP in AD or DS (average size in AD was 439 ± 49 μm², in DS 676 ± 117 μm²) (F [2, 28] = 2.73, p < 0.08). There was no trend in the 11 individuals with SP of increasing number of SP or increasing size of SP with increasing age (Fig. 2).

Alzheimer's Disease and Down Syndrome

The individuals with AD had substantially greater numbers of SP and amount of amyloid burden as compared to controls. There was a stereotypic profile of plaque size across all 15 cases, with small SP (<100 μm²) predominating. The number of SP ranged from 53–256/mm² of cortical area, with an average of 156 ± 15 (x ± SE). The amyloid burden ranged from 3.8–9.0% (6.0 ± 0.5%). However, neither SP number/mm², size of SP, nor amyloid burden correlated at all with duration of illness, giving R values of 0.0 (Fig. 3). Six of the patients had been able to take a brief neuropsychological test, the Blessed dementia scale, within 2 years of death. Analysis of these patients' scores showed no correlation of cognitive test score with SP size, number, or amyloid burden.

As expected, duration of illness was significantly correlated with Blessed dementia scale score (R = 0.73, p < 0.05). The values for SP number and amyloid burden in AD overlap with one value among the control cases, again emphasizing the difficulty in using an absolute number of SP as a strict diagnostic criterion for AD (20). The profile of SP sizes was nearly constant across all 15 cases. Figure 4 illustrates the size profile of SP in four cases across the spectrum of length of disease from 1 to 16 years.
Fig. 3. The number of SP/mm² (top) or percent of total surface area covered by Aβ immunoreactive deposits in area 20 (bottom) are plotted against duration of dementia for 15 individuals with AD. No correlation is observed.

Five individuals with DS, ages 57–65, were studied. All had substantially more SP and a greater degree of amyloid staining than either the normal elderly or the AD individuals. The number of SP averaged 252 ± 31/mm², which was significantly more than the AD group or the control group (F [2, 28] = 45.3, p < 0.0001). The amyloid burden was also much higher in DS (mean 15.6 ± 1.7%, range 10.5–19.6%) than in AD or control subjects (F [2, 28] = 75.9, p < 0.0001). However, over the 8 year span represented by these individuals, there was no tendency for either increased SP number or amyloid burden to occur with increasing age and in fact a negative correlation was found (Fig. 5).

The profile of SP size was very similar among control, AD and elderly DS subjects (Fig. 6). A logarithmic curve fits the SP profile in all three groups (R values > 0.83, p < 0.001), suggesting that similar metabolic or kinetic processes regulate SP size in all three circumstances.

DISCUSSION

The main goal of our study was to determine the natural history of amyloid deposition in brains of aged individuals and patients with AD and DS. This is an important question because several lines of evidence implicate APP and Aβ in the pathogenesis of AD. However, demonstrating a clear relationship between SP number and dementia has been difficult. Some elderly individuals who are cognitively normal have been shown to have substantial numbers of SP (20–22). In studies of AD, no relationship between the number or distribution of SP and duration or severity of dementia has been discernible in several studies (7, 8, 11, 23–25), although a weak relationship has been detected by others (26–28; see 7 or 12 for a review of these studies). The presence of neuritic plaques has been suggested as a better marker of dementia (22).

Our study tested the hypothesis that Aβ continues to accumulate during the course of AD. We used immunohistochemistry and image analysis techniques to sample amyloid SP number and calculate the total amount of amyloid staining, which we refer to as amyloid burden. In a carefully screened population of normal elderly, about half had Aβ deposits, and individuals may have a substantial number of SP that overlaps with the low end of the AD group. However, no relationship between Aβ pa-
Fig. 5. The number of SP/mm² (top) or percent of total surface area covered by Aβ immunoreactive deposits in area 20 (bottom) are plotted against age for five individuals with DS. No significant correlation with number of SP is seen, and the total amyloid burden actually appears to decline with advancing age (p < 0.05) in this group.

Fig. 6. Profile of SP size in nondemented control individuals and patients with AD and DS. The number of SP in each size class are presented as a histogram with bin sizes (in μm²) indicated on the x axis. A logarithmic fit of these data reveals significant correlations (R = 0.87, 0.83 and 0.83 for controls, AD and DS, respectively, all of which are highly significant [p < 0.001]).

there is a negative correlation. Although the age range of these individuals was fairly narrow, it nonetheless stretches over 8 years during the most severe portion of the illness.

Aβ is a nearly insoluble peptide in physiologic solution that deposits in the extracellular space as β pleated sheets. An underlying assumption in studies of SP number (or burden) is that once these deposits are formed, they remain as an insoluble deposit and accumulate in the neuropil. If this were the case, we would expect an increase in the amount of Aβ present with longer duration of illness. Alternatively, if SP undergo a dynamic “life history” of deposition, remodeling, and resorption, one could hypothesize that early in the disease SP accumulate, but later in the disease process, perhaps as there are fewer remaining neurons generating APP, resorption mechanisms lead to a steady state level.

Our results show that SP and Aβ do not tend to increase in number or size with increasing duration of illness. These data suggest that Aβ does not simply continue to accumulate in the brains of individuals with AD. Because it seems likely that Aβ continues to be generated, we suggest that these results reflect competition between deposition and resolution or remodeling of SP, leading to a "steady state" as a normal part of the pathophysiology of the illness. Biochemical studies support the concept that there may be an equilibrium of soluble and aggregated Aβ (29, 30). The steady state level of amyloid deposition is higher in DS, probably due to the gene dose effect of the APP gene on chromosome 21. While the
deposition and dissolution of Aβ may be very slow, the rate of such kinetic processes could be altered by the presence of microglia (31), astrocytes (32) or the activity of proteases (33) and protease inhibitors (34-36). It has been suggested that Aβ could be taken up by receptor-mediated uptake mechanisms (37). Additionally, our recent results suggest that Aβ may be mobilized from the neuropil by chaperone molecules such as ApoE (38). It remains for further study to determine whether all SP subtypes are equally affected by such processes, although Rozenmuller et al (35) suggest that protease inhibitors are present on SP in the cortex but not on diffuse SP in the cerebellum.

Our data are in good agreement with other reports. The degree of amyloid burden we measured, about 6%, is similar to results calculated from the study of temporal neocortex in AD individuals by Gentlemann et al (39). The amount of Aβ deposits (both diffuse and dense SP) were found to be constant in both demented and non-demented centenarians, and the density was unrelated to severity of mental deterioration (40). Berg et al (41) also found that the number of total or cored SP (detected by a modified Bielschowsky silver stain) did not correlate with degree or duration of dementia. No change in SP number was found when biopsy specimens were compared to the same individuals examined at autopsy, again supporting the view that the number of SP does not necessarily increase over the course of the disease (42).

Several alternative explanations of our observation should be considered. Our interpretation assumes that Aβ is generated at approximately the same rate in individuals with AD and that it continues to be generated throughout the course of illness. It is possible that Aβ generation occurs at different rates in different individuals or slows down in advanced disease. Alternatively, the apparent steady state could reflect saturation of some tissue factor(s) rather than active resolution of Aβ generation. Finally, although the size profile of SP did not change with increasing duration of illness, it is possible that the type of SP evolves over the course of the disease. Nonetheless, the hypothesis that Aβ deposits are generated, remodeled, and dissolved over the lifetime of the illness may help explain the paradox that while Aβ is integrally associated with the genetics and histopathology of AD, the absolute number of SP or amount of Aβ present at a given time does not correlate well with duration or severity of illness (12, 41). Understanding processes by which Aβ deposits resolve could lead to additional targets for therapeutic intervention in AD.

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